Postmortem Diagnosis of Rabies in Animal Brain by Fluorescent Antibody Testing

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***Abstract*—**There are different protocols to diagnosis rabies infection in animals and different tissues. In this laboratory study we used a postmortem diagnosis of rabies in animal brain by fluorescent antibody testing (FAT). The samples were prepared on glass slides and fluorescent antibody staining was used to detect Negri bodies in mouse brain to confirm rabies infection. Existing green particles in microscopic observation indicated positive result for existing Negri bodies and rabies infection.

***Keywords*—** Rabies, Animal brain, FAT.

I. INTRODUCTION

RABIES virus, a rhabdovirus of the Lyssavirus genus, is a negative-strand virus containing a nonsegmented RNA molecule. Like vesicular stomatitis virus (VSV), the model system for rhabdoviruses, rabies virus has a bullet shaped morphology [1],[2]. The most studied rhabdovirus, the RV particle contains five proteins, produced from five capped, methylated, polyadenylated viral mRNAs. Three of these viral proteins, the nucleoprotein (N), the phosphoprotein (P) and the RNA polymerase (L), form a helical ribonucleoprotein complex (RNP) in association with the genomic RNA. It is the N protein that directly encapsidates the viral genome, and the RNP is condensed into a coiled helical structure by the matrix protein (M). [3] – [5].

Most animals can be infected by the virus and can transmit the disease to humans. Infected bats, monkeys, raccoons, foxes, skunks, cattle, wolves, coyotes, dogs, mongooses (normally yellow mongoose) and cats present the greatest risk to humans. [6] – [8].

The virus is usually present in the nerves and saliva of a symptomatic rabid animal. [9],[10]. The route of infection is usually, but not always, by a bite. [11]. Transmission between humans is extremely rare. [12]. After a typical human infection by bite, the virus enters the peripheral nervous system. It then travels along the afferent nerves toward the central nervous system. [13]. During this phase, the virus cannot be easily detected within the host, and vaccination may still confer cell-mediated immunity to prevent symptomatic rabies. When the virus reaches the brain, it rapidly causes encephalitis, the prodromal phase, and is the beginning of the symptoms. Rabies can be difficult to diagnose, because, in the early stages, it is easily confused with other diseases or with aggressiveness [14]. The reference method for diagnosing rabies is the fluorescent antibody test (FAT, a immunohistochemistry procedure), which is recommended by the World Health Organization (WHO) [15]. In this laboratory study we used fluorescent staining and tested on mouse brain to detect Negri bodies.

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II. MATERIAL AND METHODS

In our method we removed and prepared brain samples of infected mice with rabies. Because rabies is present in nervous tissue (and not blood like many other viruses), the ideal tissue to test for rabies antigen is brain. After preparation of brain samples, they were fixed with acetone cold for half an hour. Then 100 ml nucleocapsid antibodies conjugated with FITC was added and then incubated at 37 ° C for one hour . After washing with PBS and drying , the slides were observed by using a fluorescence microscope. In this way, when labeled antibody was incubated with rabies-suspect brain tissue, it would bind to rabies antigen. Unbound antibody was washed away and areas where antigen was present could be visualized as fluorescent-apple-green areas showing Negri. (green) bodies confirming the existence of rabies in tissue.

III. RESULTS

Figure I and II green bodies (Negri bodies) in brain tissue observed by using fluorescence microscope confirming the rabies infection.

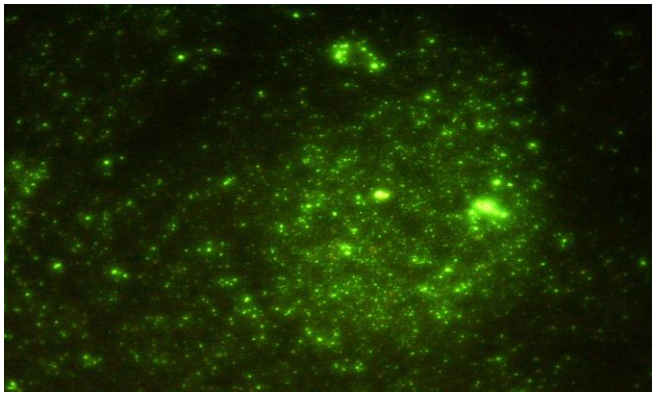

Fig.1. Negri Bodies In Mice Brain Tissue Infected With Rabies Virus

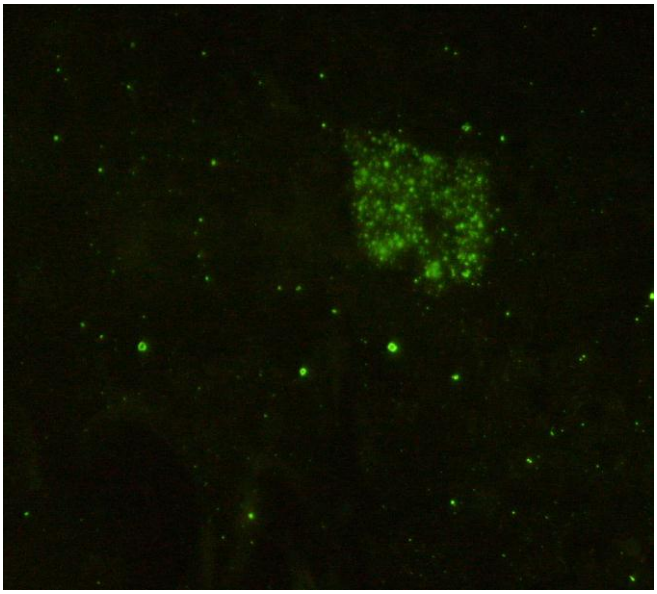

Fig. 2. Negri Bodies In Mice Brain Tissue Infected With Rabies Virus.

IV. Discussion

Our findings showed that the staining method used in our research center was effective in ddiagnosis of rabies infection. Other studies also show that the direct immunofluorescent-antibody (dIFA) test for rabies virus antigen in brain tissue is the preferred test for rabies diagnosis [15]. Immunoperoxidase tests of formalin-fixed brain material or dIFA tests of proteinase-digested fixed brain material have been developed but have not been thoroughly evaluated for sensitivity. Virus isolation is not performed for routine diagnostic tests but is useful when the results of the dIFA are inconclusive or unusual. Since rabies virus is not cytopathic, evidence of virus growth is obtained by dIFA detection of viral antigen in acetone- fixed cell monolayers of either mouse neuroblastoma or baby hamster kidney cell lines[16]. Because the dIFA test is rapid, sensitive, specific, easy to perform, and relatively inexpensive, molecular techniques such as PCR or hybridization probes are not used for routine rabies diagnosis. However, molecular techniques have been useful in antemortem diagnosis[17].

V. Conclusion

Our findings show that the protocol used in our study to diagnose rabies was effective and applied method.

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